mixture. The NMR spectrum for the aqueous layer showed signals exclusively attributable to BisNA$^+$($C_6$,Bzl), the yield being quantitative as confirmed by HPLC analysis.

The reaction behavior was monitored by $^1$H NMR spectroscopy. Into a solution of BisNAH($C_6$,Bzl) (25 mg, $5.0 \times 10^{-5}$ mol) in deuteriochloroform (200 μL), containing 1% acetonitrile as an internal reference in an NMR tube, was added an equimolar amount of hexachloroacetone (7 μL, $5.0 \times 10^{-5}$ mol), i.e., the ketone in 0.5 equiv to the dihydronicotinamide unit. After the reaction mixture was allowed to stand at room temperature for 2 h, $^1$H NMR signals for BisNAH($C_6$,Bzl) disappeared completely on one hand and a proton signal attributable to the 2-proton of the alcohol product (4.81 ppm downfield from $Me_4Si$) was observed on the other. The signal intensity indicated quantitative formation of the alcohol. The reaction of (*N*-Et)BNAH (44 mg, $1.8 \times 10^{-4}$ mol) with the ketone (13.5 μL, $9.0 \times 10^{-5}$ mol) was carried out in a similar manner in deuteriochloroform (250 μL), containing 1% acetonitrile, at room temperature. The resulting NMR spectrum indicated that the alcohol product was formed quantitatively, and a half of the initial amount of (*N*-Et)BNAH was consumed.

**Kinetic Measurements.** Each run was initiated by injecting hexachloroacetone (4.5 μL, $3.0 \times 10^{-5}$ mol) into a solution (3 mL) of an appropriate 1,4-dihydronicotinamide, which was preequilibrated at 25.0 ± 0.1 °C in a thermostated 1-cm quartz cell set in the spectrophotometer. The initial concentrations of dihydronicotinamides were maintained constant at $1.0 \times 10^{-4}$ M for monodihydronicotinamides and at $5.0 \times 10^{-5}$ M for bis(dihydronicotinamide)s, so that the total concentrations in terms of the dihydronicotinamide unit were adjusted at $1.0 \times 10^{-4}$ M. Progress of the reaction was monitored spectrophotometrically by measuring the absorbance decay at 350 nm, which is referred to consumption of the dihydronicotinamide moiety. Control experiments indicated that no reaction took place in the absence of the ketone. Hexachloroacetone did not undergo any spontaneous decomposition in the solvents employed here, as confirmed by electronic spectroscopy. For kinetic runs under anaerobic conditions, all the solutions placed in a specially designed cell were purged with argon just before injection of the ketone.

**Registry No. 1**, 10400-19-8; **2a**, 39642-79-0; **2b**, 73041-73-3; **2c**, 77091-29-3; **2d**, 77091-31-7; **3**, 4314-66-3; **4**, 51055-31-3; BisNA$^+$($C_4$,Bzl), 82352-73-6; BisNA$^+$($C_6$,Bzl), 81408-01-7; BisNA$^+$($C_8$,Bzl), 82352-74-7; BisNA$^+$($C_{10}$,Bzl), 82352-75-8; BisNA$^+$(Et,*p*-xyl), 82352-76-9; BisNA$^+$(Et,*m*-xyl), 82352-77-0; BisNA$^+$(Et,*o*-xyl), 82352-78-1; cBisNA$^+$($C_4$,*p*-xyl), 82352-79-2; cBisNA$^+$($C_6$,*p*-xyl), 82352-80-5; cBisNA$^+$($C_6$,*p*-xyl), 82352-81-6; BisNA$^+$($C_6$,Pr), 82352-82-7; BisNA$^+$(Pr,$C_6$), 82352-83-8; cBisNA$^+$($C_6$,$C_6$), 82352-84-9; (*N*-Et)BNA$^+$, 81388-57-0; (*N*-Pr)PNA$^+$, 82352-85-0; (*N*-Et-l)BNA$^+$, 82352-86-1; PNAH, 17750-24-2; NAH-$C_6$-Ad, 27474-83-5; BisNAH($C_4$,Bzl), 78857-79-1; BisNAH($C_6$,Bzl), 78844-34-5; BisNAH($C_8$,Bzl), 78844-35-6; BisNAH($C_{10}$,Bzl), 78844-36-7; BisNAH(Et,*p*-xyl), 78844-37-8; BisNAH(Et,*m*-xyl), 82352-87-2; BisNAH(Et,*o*-xyl), 82352-88-3; cBisNAH($C_4$,*p*-xyl), 82352-89-4; cBisNAH($C_6$,*p*-xyl), 78857-80-4; cBisNAH($C_4$,*o*-xyl), 82352-90-7; *N*-EtBNAH, 78844-38-9; BisNAH($C_6$,Pr), 82352-91-8; BisNAH(Pr,$C_6$), 82352-92-9; cBis($C_6$,$C_6$), 82352-93-0; (*N*-Et-I)PNAH, 82352-94-1; (*N*-Et-l)BNAH, 82352-95-2; α,ω-diaminobutane, 110-60-1; α,ω-diaminohexane, 124-09-4; α,ω-diaminooctane, 373-44-4; α,ω-diaminodecane, 646-25-3; benzyl chloride, 100-44-7; *p*-xylylene dichloride, 623-25-6; *o*-xylylene dibromide, 91-13-4; *n*-propyl iodide, 107-08-4; 1,6-diiodohexane, 629-09-4; hexachloroacetone, 116-16-5.

# Carrier-Mediated Transport of Amino Acid and Simple Organic Anions by Lipophilic Metal Complexes

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**Abstract:** A variety of lipophilic metal complexes were examined as a new type of anion-transport carrier in a methylene chloride liquid membrane system. Some copper complex carriers, composed of neutral ligands, could effectively mediate active and passive transport of simple organic anions and amino acid derivatives. Their transport properties were largely different from those of previously reported organic anion carriers and were essentially dependent on the combined characteristics of ligand molecule, coordinated metal ion, antiport anion, and others. Liquid–liquid extraction experiments of each elementary process clearly demonstrated that the overall transport rate was mostly determined by that of the substrate-releasing process from the membrane. Active transport of biologically important amino acid derivatives was successfully achieved by this type of carrier, providing a new chemical analogue to some biological transport systems.

Membrane transport is a fundamental and essential process in many biological systems, and its model systems have actively been studied. Of particular, many kinds of synthetic carriers such as crown ethers[1] and cryptands[2] have been utilized as potential carriers for transporting alkali metal and organic ammonium cations and permitted the successful resolution of some racemates into the optically active forms.[3] In marked contrast, little attention has been directed toward transport of anionic species such as amino acids (carboxylate, phenolate, and thiolate anions) and ATP (phosphate anion), which are important from the biochemical and medical points of view. Hence, development of a new type of carrier capable of transporting these organic anions is required not only to simulate many biological systems but also to create a new methodology in separation science.

Recently some host molecules have been prepared,[4] but we know of only a few successful examples of carrier-mediated transport of organic anions.[5] Lehn et al. and Tabushi et al. have presented

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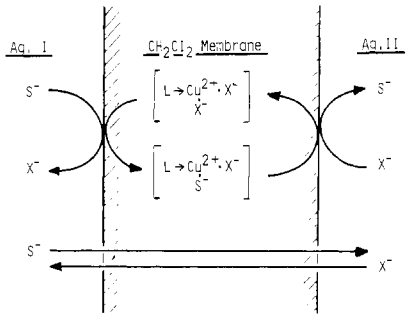


**Figure 1.** Liquid membrane system for transport of organic anions: $S^-$, organic anion; $X^-$, antiport anion; L, neutral ligand.

typical examples in which the quaternary ammonium salts form lipophilic complexes with guest anions and transport them. Shinbo et al. and other investigators have reported redox-reaction-driven transference systems, where the redox compounds such as tetramethyl-*p*-phenylenediamine and dibutylferrocene serve as both an electron carrier and anion carrier.

More recently we communicated that[6] lipophilic copper complex **1**, employing some complicated ligand, $[CH_2CH_2N(CSNHPh)]_{n=8}$, transported several simple organic anions with unique specificities. This copper complex was able to bind and carry organic anions via "substrate anion–copper ion–ligand" ternary complex formation. In such a ternary complex,[7] ligand–substrate anion interaction can be effectively cooperated with metal ion–substrate anion interaction and contribute to create unique and higher "substrate specificity", as known for example for enzyme–metal ion–substrate complexes. Therefore, a variety of transition-metal complexes, capable of forming ternary complexes, are expected to be a new class of anion carriers displaying characteristic functions not found in the previously reported carrier systems.

The present paper describes the detailed results of metal complex carrier-mediated transport systems. Although several polyethers have been reported to carry anionic species as counteranion together with alkali metal cations,[8] we believe that the present system is the first successful example of transition-metal complex carriers which transport organic anions with excellent efficiencies. Some kinds of transition-metal complexes were examined and shown to mediate active and passive transport of a variety of organic anions and amino acid derivatives. We found that interesting and important transport phenomena could, in principle, be achieved by the appropriate choice of ligand molecule, coordinated metal ion, antiport anion, etc. Moreover, the results obtained would provide a new possibility in modeling anion transport phenomena across the biomembrane, as well as further applications to anion separation and related processes.

## Results and Discussions

**Liquid Membrane System.** The liquid membrane system used in this study is shown schematically in Figure 1. The examined

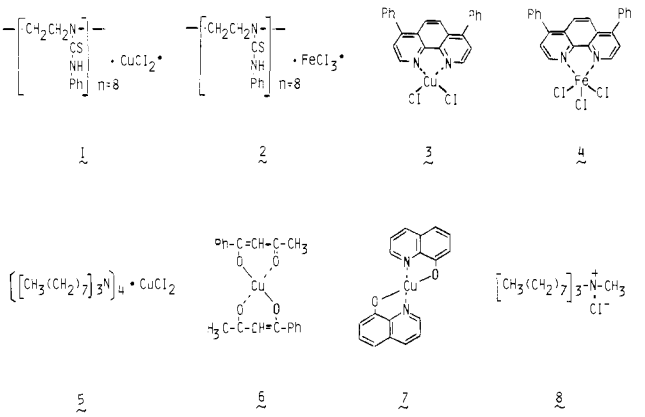


**Figure 2.** Structure of examined carrier for anion transport; (*) complex compositions; see text.

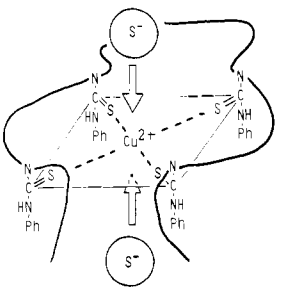


**Figure 3.** Schematic sketch of "substrate anion–carrier **1**" complex. $S^-$, substrate anion.

metal complexes **1**–**7** (see Figure 2) are much less soluble in the aqueous phases I and II than in the methylene chloride liquid membrane. We confirmed that the leakages of metal ions from the membrane into aqueous phases were negligible unless noted otherwise. At the interface of aqueous phase I and the methylene chloride membrane, lipophilic metal complex binds organic anion substrate ($S^-$) via coordinated anion exchange and carries it through the membrane. Then, the coordinated anion ($S^-$) is exchanged by antiport anion ($X^-$) and released into aqueous phase II. The net result is that organic anion ($S^-$) and antiport anion ($X^-$) appear simultaneously in aqueous phases II and I, respectively. Therefore, this process can be considered as an artificial model of biological antiport transport systems.[9] Although some surfactants such as **8** have been reported to transport carboxylate anions in a similar fashion by Lehn et al.,[5a] a new type of metal complex carrier was applied.

**Passive Transport of Simple Organic Anions.** Three types of lipophilic metal complexes (linear multidentate ligand–, aromatic amine–, and aliphatic amine–metal complexes) and organic surfactant (ammonium salt) were examined as anion carriers for passive transport of simple organic anions.

The metal complexes **1** and **2** contain neutral ligand having the structure $[CH_2CH_2N(CSNHPh)]_{n=8}$. This linear multidentate ligand was prepared by ring-opening oligomerization of 1-(*N*-phenylthiocarbamoyl)aziridine[10] and has a regular recurrence of metal coordination site (NCSNH) and hydrophobic moieties (Ph, $CH_2CH_2$) in a single chain. As reported before,[10b] some metal ions were coordinated with this type of ligand molecule in a manner of the pseudocyclic ligand and made soluble in the lipophilic media. In its copper complex, four thiocarbamoyl sulfur atoms of the ligand can be located in a square-planar configuration, and the periphery of the metal complex consists of hydrophobic moieties making an exohydrophobic complex[11] (see Figure 3). These

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Table I. Carrier-Mediated Transport of Simple Organic Anions[a]

| substrate anion | antiport anion | transport rate × $10^6$, mol/h[b] 1 | 2[c] | 3 | 4 | 5 | 8 |
|---|---|---|---|---|---|---|---|
| $p$-$O_2NC_6H_4O^-$ | $OH^-$ [d] | 0.35 | | | | | 0.13 |
| | $Cl^-$ | 8.12 | 2.45 | 8.39 | 0.46 | 3.81 | 2.42 |
| | $ClO_4^-$ | 26.57 | | | | | 13.20 |
| | $SCN^-$ | 15.94 | | | | | 30.41 |
| $m$-$O_2NC_6H_4O^-$ | $Cl^-$ | 4.62 | 3.40 | 1.21 | 1.25 | 0.31 | 3.98 |
| $o$-$O_2NC_6H_4O^-$ | $Cl^-$ | 3.55 | 0.15 | 0.29 | 0.40 | 0.28 | 5.00 |
| $o,o'$-$(O_2N)_2C_6H_3O^-$ | $Cl^-$ | 5.95 | | | | | 0.42 |
| $o,o',p$-$(O_2N)_3C_6H_2O^-$ | $Cl^-$ | 2.62 | | | | | 0.05 |
| $p$-$O_2NC_6H_4CO_2^-$ | $Cl^-$ | 26.50 | 6.63 | 8.29 | 1.06 | 2.38 | 9.40 |
| $m$-$O_2NC_6H_4CO_2^-$ | $Cl^-$ | 22.22 | 8.44 | 9.38 | 1.30 | 3.67 | 12.65 |
| $o$-$O_2NC_6H_4CO_2^-$ | $Cl^-$ | 28.33 | 21.77 | 8.81 | 0.98 | 4.03 | 16.25 |
| $p$-$O_2NC_6H_4PO_4^{2-}$ | $Cl^-$ | 21.40 | 4.01 | 8.29 | 0.49 | 5.91 | 15.82 |
| $m$-$O_2NC_6H_4SO_3^-$ | $Cl^-$ | 23.57 | 11.59 | 3.24 | 1.76 | 4.26 | 2.52 |

[a] Initial concentrations: aqueous phase I—sodium salt of substrate, 0.4 mmol/$H_2O$, 4 mL (pH 7–10); aqueous phase II—sodium or potassium salt of antiport anion, 5.0 mmol/$H_2O$, 10 mL; organic phase—carrier, 0.037 mmol/$CH_2Cl_2$, 8 mL. [b] Reproducibility, ±10% or better. [c] Carrier 2, 0.030 mmol/$CH_2Cl_2$, 8 mL. The values indicated were normalized. [d] No salt of antiport anion was added into aqueous phase II.

Table II. Extraction of Simple Organic Anions

| substrate anion | system[a] | extraction % in $CH_2Cl_2$ 1 | 8 |
|---|---|---|---|
| $p$-$O_2NC_6H_4O^-$ | A | 41 | 53 |
| | B | 91 | 62 |
| $m$-$O_2NC_6H_4O^-$ | A | 34 | 63 |
| | B | 79 | 53 |
| $o,o'$-$(O_2N)_2C_6H_3O^-$ | A | 52 | 62 |
| | B | 96 | 85 |
| $p$-$O_2NC_6H_4CO_2^-$ | A | 25 | 54 |
| | B | 48 | 33 |
| $p$-$O_2NC_6H_4PO_4^{2-}$ | A | 8 | 17 |
| | B | 12 | 4 |
| $m$-$O_2NC_6H_4SO_3^-$ | A | 19 | 62 |
| | B | 4 | 76 |

[a] System A: carrier (0.0186 mmol) in $CH_2Cl_2$ (4 mL), substrate (0.0186 mmol) in 0.025 N aqueous NaOH (4 mL). System B: carrier (0.0186 mmol) in $CH_2Cl_2$ (4 mL), substrate (0.0186 mmol) in 0.5 N aqueous KCl (4 mL). In each system, after 1 h stirring at room temperature, substrate concentration in the aqueous phase was determined by spectroscopic method.

structural features of the complex allow substrate anion to be situated at a relatively longer distance along the central axis perpendicular to the square and attracted into the hydrophobic cavity. Hence, this type of metal complex is expected to display sufficient stabilities to facilitate anion exchange at interfaces; the outer surface may be lipophilic so that the complex can be soluble in the membrane.

Bathophenanthroline–metal complexes **3** and **4**, which have often been utilized as model complexes of biological activities, were chosen as more simple metal complex carriers. Their binary complexes have been well characterized, and their ternary complexes may be highly stabilized by $\pi$ back-bonding from the metal ion to the ligand molecule.[12] Aliphatic amine–copper complex, tetrakis(trioctylamine)copper(II) chloride (**5**), was also examined as a membrane carrier. Since these metal complexes have some different structural features (steric/electronic environments around the metal ion), they would display newer and characteristic transport functions. For a reference, trioctylmethylammonium chloride (**8**), which had already been reported to be an effective anion carrier,[5a] was employed.

As substrate anions, we chose a series of organic anions having almost the same molecular sizes and various negatively charged groups: phenolate, sulfonate, carboxylate anions, and phosphate dianion. The passive transport experiments were performed by using a similar apparatus as described before,[6,13] (see the Experimental Section). The concentrations of each substrate anion in the aqueous phase II were determined by spectroscopic methods, and the initial rates obtained are listed in Table I.

The metal complexes **1–5** transported a variety of organic anions with respective efficiencies. Among them, the copper complex **1** showed higher transport rates than other examined metal complexes and conventional organic surfactant carrier. For example, carrier **1** transported the $p$-nitrophenolate anion in an amount equal to 22% of the total after 12 h and the chloride anion in the opposite direction. The transport rates of the $p$-nitrophenolate anion increased by using perchlorate or thiocyanate anion as the antiport anion. Probably, they may readily release anionic substrate bound to the carrier. A similar effect of antiport anion has been observed in the carrier-mediated transport of ADP by Tabushi et al.[5b]

The copper complex carriers **3** and **5** could also effectively mediate passive transport of some kinds of organic anions such as phenolate and sulfonate anions, even though their transport rates were generally lower than those of complex **1**. The fact that metal complexes including simple and ordinary ligands transported organic anions suggests a potential utilization of a number of metal complexes to the anion transport systems. A deeper insight into the relationship between ligand structure and transport ability should be required to design a more specific and powerful carrier.

Iron complexes **2** and **4** had somewhat lower transport abilities for examined organic anions compared with the corresponding copper complexes **1** and **3**, indicating that the nature of coordinated metal ions should be considered as an essential factor for determining the transport efficiencies.

Carrier **1** exhibited a remarkable and unique transport selectivity sequence: phenolate anion < phosphate dianion ≪ carboxylate anion ~ sulfonate anion. This transport trend of metal complex carrier **1** was quite different from that displayed by surfactant carrier **8**: sulfonate anion ~ phenolate anion < carboxylate anion < phosphate dianion. Particularly, carrier **1** had great advantages in the transport of 2,6-dinitrophenolate, picrate, and nitrobenzenesulfonate anions, which were slowly transported by carrier **8**.

Table I demonstrates that metal complexes **2–5** exhibited characteristic transport specificities, suggesting that vast regulation of the transport selectivities and efficiencies could be attained by the appropriate choice of ligand molecule, central metal ion, antiport anion, and their combinations. More elaborate modifications of ligand structure and proper choice of metal ion would enable us to have a specific anion carrier complex.

In order to determine the nature of the present transport system, we performed liquid–liquid extraction experiments in which some organic anions are distributed between aqueous NaOH solution (or aqueous KCl solution) and carrier-containing methylene chloride solution. The organic anions are complexed with carrier and extracted into the phase of methylene chloride, and the degrees of extraction are taken to be a measure of the complexing ability of the carrier for each substrate anion. The results are shown in Table II.

Of the anions examined, carboxylate, sulfonate, and phosphate anions, which were efficiently transported by carrier **1**, were found to be relatively loosely complexed with copper complex **1**. On the other hand, phenolate anions, transported with lower rates, were shown to be accumulated into the methylene chloride phase as stable "phenolate anion–carrier complex". A parallel obser-

(11) Earlier laser Raman and far-IR spectroscopic studies on the related copper complexes have indicated that copper ion is bound to the thiocarbamoyl sulfur atoms. Electronic and CD spectral changes also suggested $Cu-S_4$ complex formation in a square-planar fashion. See: Araki, T.; Tsukube, H. *Macromolecules* **1978**, *11*, 250–255.

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Table III. Absorption Maxima of Bound Phenolate Anions[a]

| substrate anion | absorption maxima, nm in $H_2O$, no carrier | in $CH_2Cl_2$ 1 | in $CH_2Cl_2$ 8 |
|---|---|---|---|
| $p$-$O_2NC_6H_4O^-$ | 400 | 411 | 418 |
| $o,o'$-$(O_2N)_2C_6H_3O^-$ | 430 | 453 | 463 |

[a] Conditions: see Table II, system A.

vation was observed in the carrier **8** system. Carrier **8** was confirmed to transport carboxylate and phenolate anions with higher transport rates, which were not so tightly bound to the carrier. These results demonstrate that overall transport rate is dependent on that of release rather than that of extraction in the present system. Several investigators have also observed similar relationships between complexing ability and transport efficiency of the carrier in cation-transport experiments.[1,2,14] It has been established that a host molecule giving a modestly stable complex with a guest species is an effective carrier. The same conclusion could be drawn in the present anion-transport system.

The electronic spectra of organic anion–carrier complexes provided further information on the coordinated states of substrate anions. By equilibrating a methylene chloride solution of carrier **1** or **8** with aqueous phenolate anion solution, some absorption bands assignable to the carrier-bound phenolate anions appeared in the electronic spectra. Table III summarizes the absorption maxima of these bands, showing that they were shifted to longer wavelengths than those observed in the aqueous solution. Larger spectral shifts were found in the "ion-pair complex" of carrier **8**–phenolate anion than in the "coordination complex" of carrier **1**–phenolate anion. Although not yet fully characterized, the nature of phenolate anion–carrier complexes would determine the coordination states, and phenolate anions may be bound to carrier **8** more strongly than to carrier **1**. The methylene chloride membrane solution during the transport experiments exhibited similar spectral profiles, supporting the complex formation of "substrate anion–copper complex carrier" as transient species, as shown in Figure 1. In the square-planar copper complexes like carrier **1**, the axial ligation is well-known to be relatively weak and so called "labile".[12a] Such coordination properties of metal complex carrier **1** can lead to the effective formation of a transient ternary complex with a suitable stability and to the characteristic transport abilities of unique transport selectivity and high efficiency.

**Passive Transport of Amino Acid Derivatives as Carboxylate Anions.** We applied a new type of anion carrier to the transport of biologically important amino acid derivatives,[9] since transport behaviors of novel metal complex carriers as described above are expected to attain further interesting functions such as selective and active transport.

The five lipophilic metal complexes **1**, **3**, **5**, **6**, and **7**, and trioctylmethylammonium chloride (**8**) for comparison, were studied as anion carriers. The passive transport experiments of amino acid anions were carried out in a similar manner as mentioned above. The initial rates shown in Table IV were obtained from the rates of appearance of amino acid anions in aqueous phase II. No diffusion was detected in the absence of carrier.

The copper complex **1** transported a variety of *N*-benzoyl amino acid and dipeptide derivatives with comparable efficiencies to those of organic anion carrier **8**.[5a] Under the employed conditions (see Table IV), carrier **1** transported *N*-benzoylalanine in an amount equal to 57% of the total after 8 h and chloride anion (antiport anion) in the opposite direction. Such a fast transport rate was hardly observed in the presence of ligand molecule alone, indicating that anion binding of the metal cation site in the carrier complex seemed to play an important role. As we confirmed that the leakages of copper ion from membrane into aqueous phases were usually small,[15] the copper complex could be circulated as a carrier,

Table IV. Carrier-Mediated Transport of Amino Acid Derivatives[a]

| substrate | transport rate[b] × $10^6$, mol/h 1 | 3 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|
| Bz-Gly | 21.3 | 10.5 | 13.6 | 0.2 | 0.4 | 21.6 |
| Bz-Ala | 18.5 | 7.0 | 16.1[e] | 0.1 | 0.4 | 14.6 |
| Bz-Glu | 14.4[c] | 8.9 | 13.8 | 0.3[e] | 0.1 | 21.6 |
| Bz-Gly-Gly | 14.0[c] | 18.2 | 10.9 | 0.3 | 0.1 | 20.4 |
| Bz-Met | 11.5 | 3.6 | 5.4[e] | 0.1 | 0.1 | 14.4 |
| Bz-Val | 9.5 | 4.8 | 11.8[e] | 0.1 | 0.1 | 10.0 |
| Bz-Leu | 9.5 | 3.7 | 4.6[e] | 0.1 | 0.3 | 12.7 |
| Bz-Phe | 5.4 | 2.4 | 2.7[e] | 0.1[e] | 0.1 | 6.1 |
| Ala | 2.1[d] | | | | | 5.2 |
| Phe | 1.2[d] | | 1.3[d] | 2.2[d] | 1.5 | 16.4 |

[a] Conditions: aqueous phase I–substrate, 0.3 mmol/0.1 N aqueous NaOH, 3 mL: aqueous phase II–KCl, 5.0 mmol/$H_2O$, 9 mL; membrane–carrier, 0.037 mmol/$CH_2Cl_2$, 8 mL. [b] Reproducibility: ±15% or better. [c] Small amount of copper ion was moved into aqueous phase I. [d] Leakage of copper ion was not negligible. [e] Copper species was partially suspended.

as shown in Figure 1. It was noted that considerable amounts of copper ion were extracted into the aqueous phases in the transport of nonsubstituted phenylalanine and alanine. When these bidentate ligands were chosen as substrate anion, the copper ion was leaked into the aqueous phase by complexation with amino acids, and the transport rates were modestly suppressed. Therefore, the use of carrier **1** appeared to be limited to the transport of monodentate anionic substrates such as N-substituted amino acids.

Carrier **1** showed a quite interesting transport trend for a series of amino acid derivatives examined: Bz-Gly > Bz-Ala > Bz-Glu ~ Bz-Gly-Gly > Bz-Met > Bz-Val ~ Bz-Leu > Bz-Phe > Ala ~ Phe. This is remarkably different from those of reported systems. Some investigators[5a,16] have reported that the transport rates of nonsubstituted amino acids were mainly proportional to the hydrophobicities of the substrates, suggesting that the extraction process of the substrates into the membrane could be importantly operating: Phe > Leu > Ala > Gly. Introduction of hydrophobic and bulky benzoyl groups into the substrates had great influences on the transport phenomena.

The transport selectivities shown by the small copper complex **3** were of interest: Bz-Gly-Gly > Bz-Gly > Bz-Glu > Bz-Ala > Ba-Val > Bz-Met ~ Bz-Leu > Bz-Phe. Bz-Gly-Gly and Bz-Glu anions were effectively transported by copper complex **3**, though carrier **3** generally showed a low transport rate. Probably additional metal coordination of functional groups ($CO_2H$, NHCO) in these substrates would be significantly participating in the carrier–substrate complex formation.

Tetrakis(trioctylamine)copper(II) chloride (**5**) also transported amino acid anions as carboxylate anions. When it was employed as carrier, large amounts of the copper species were deposited on the wall of transport cell, and its transport abilities were lower than those of **1**. This suggests that the higher lipophilicity of the formed ternary complex can essentially enhance the transport rate.

As opposed to complexes **1**, **3**, and **5**, anionic ligand coordinated complexes **6** and **7** were found to barely transport amino acid anions. In the latter complexes, where the positive charges of the central metal ion were neutralized by the coordinated ligand anions, amino acid anions could not be involved in further coordination. These results clearly indicate that neutralization of charge is one of the important driving forces leading to ternary complex formation. In other words, the formation of lipophilic "substrate anion–metal ion–neutral ligand" ternary complex is an essential factor in designing a useful metal complex carrier.

**Active Transport of Amino Acid Derivatives by Metal Complex Carriers.** Active transport of amino acid derivatives was attempted by using a U-shaped apparatus of a "Pressman cell" (see Ex-

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(15) Under the employed conditions (see Table IV), ca. 7% of copper ion initially added was leaked from membrane into aqueous phases after 12 h of operation.

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Table V. Active Transport of Amino Acid Anions by Carriers 1, 3, and 8[a]

| substrate | antiport anion, mmol | equilibrated substrate distribution, mmol[b] aq I | membrane | aq II |
|---|---|---|---|---|
| | Carrier 1 System | | | |
| Bz-Gly | (0) | 0.219 | 0.062 | 0.219 |
| | $Cl^-$ (1.0) | 0.216 | 0 | 0.284 |
| | $Cl^-$ (2.5) | 0.122 | 0.005 | 0.373 |
| | $Cl^-$ (5.0)[c] | 0.107 | 0.005 | 0.388 |
| | $Cl^-$ (10.0) | 0.091 | 0.047 | 0.362 |
| | $ClO_4^-$ (5.0) | 0.124 | 0.033 | 0.343 |
| | $SCN^-$ (10.0) | 0.109 | 0.053 | 0.338 |
| Bz-Ala | $Cl^-$ (2.5) | 0.170 | 0.060 | 0.270 |
| | $Cl^-$ (5.0) | 0.082 | 0.029 | 0.389 |
| Bz-Glu | $Cl^-$ (5.0) | 0.147 | 0.033 | 0.320 |
| Bz-Gly-Gly | $Cl^-$ (5.0) | *d* | *d* | *d* |
| Bz-Met | $Cl^-$ (5.0) | 0.152 | 0.045 | 0.303 |
| Bz-Val | $Cl^-$ (5.0) | 0.157 | 0.073 | 0.270 |
| Bz-Leu | $Cl^-$ (5.0) | 0.159 | 0.082 | 0.259 |
| Bz-Phe | $Cl^-$ (2.5) | 0.206 | 0.044 | 0.250 |
| | $Cl^-$ (5.0) | 0.143 | 0.105 | 0.252 |
| | Carrier 3 System | | | |
| Bz-Gly | (0) | 0.210 | 0.080 | 0.210 |
| | $Cl^-$ (1.0) | 0.194 | 0.030 | 0.276 |
| | $Cl^-$ (5.0) | 0.134 | 0.034 | 0.332 |
| | $Cl^-$ (10.0) | 0.098 | 0.069 | 0.333 |
| | $ClO_4^-$ (5.0) | 0.156 | 0.067 | 0.277 |
| | $SCN^-$ (5.0) | 0.194 | (0.049)[e] | 0.257 |
| Bz-Ala | $Cl^-$ (5.0) | 0.049 | 0.090 | 0.361 |
| Bz-Glu | $Cl^-$ (5.0) | 0.079 | 0.053 | 0.368 |
| Bz-Gly-Gly | $Cl^-$ (5.0) | 0.059 | 0.024 | 0.417 |
| Bz-Met | $Cl^-$ (5.0) | 0.176 | 0.092 | 0.232 |
| Bz-Val | $Cl^-$ (5.0) | 0.146 | 0.104 | 0.250 |
| Bz-Leu | $Cl^-$ (5.0) | 0.180 | 0.090 | 0.230 |
| Bz-Phe | $Cl^-$ (5.0) | 0.207 | 0.083 | 0.210 |
| | Carrier 8 System | | | |
| Bz-Gly | $Cl^-$ (5.0) | 0.083 | 0.003 | 0.414 |
| Bz-Ala | $Cl^-$ (5.0) | 0.069 | 0.001 | 0.430 |
| Bz-Glu | $Cl^-$ (5.0) | 0.117 | 0.017 | 0.366 |
| Bz-Glu-Gly | $Cl^-$ (5.0) | 0.112 | 0.021 | 0.367 |
| Bz-Met | $Cl^-$ (5.0) | 0.184 | 0.037 | 0.284 |
| Bz-Val | $Cl^-$ (5.0) | 0.117 | 0.021 | 0.362 |
| Bz-Leu | $Cl^-$ (5.0) | 0.165 | 0.049 | 0.286 |
| Bz-Phe | $Cl^-$ (5.0) | 0.214 | 0.041 | 0.245 |

[a] Initial concentrations: aqueous phase I—substrate, 0.25 mmol/0.05 N NaOH, 5 mL; aqueous phase II—substrate, 0.25 mmol, salt of antiport anion, 0–10 mmol/0.05 N NaOH, 5 mL; membrane—carrier, 0.056 mmol/$CH_2Cl_2$, 12 mL. [b] The concentrations of substrate in both aqueous phases were determined spectroscopically after 24 h. [c] 0.053 mmol of Bz-Gly was actively transported after 12 h in this system, while 0.060 mmol of Bz-Gly was found to be passively transported in the absence of substrate in aqueous phase II. [d] Considerable amounts of copper ion were eluted into the aqueous phases. [e] The amounts of precipitates were not negligible in this case.

perimental Section), in which initial concentrations of each substrate and pH conditions were the same for both aqueous phases separated by the methylene chloride membrane. The concentrations of substrate in both aqueous phases were followed spectroscopically, and apparently equilibrated concentrations, determined usually after 24 h, are listed in Table V.

In the absence of antiport anion (chloride, thiocyanate, and perchlorate anions) in aqueous phase II, substrate anion was simply accumulated into the membrane phase but not transported. When the antiport anion was added, amino acid derivatives were successfully transported against their concentration gradients by aid of copper complex **1** or **3** or surfactant **8**. Although a chemical reaction was not linked with the transport process, so-called "uphill" transport of amino acid anions could be achieved. For *N*-benzoylglycine as a typical example, the actively transported amounts were found to be enhanced by increasing chloride anion (antiport anion) concentrations in aqueous phase II, showing that coupling to the antiport anion gradient was used to pump the amino acid anions up. The utilization of thiocyanate or perchlorate anion as antiport anion had only slight influences on the apparently equilibrated amounts of each substrate, even though initial rates were really accelerated. The transport experiment conducted under the conditions in which aqueous phase II initially contained chloride anion alone shows that the presence of amino acid anion in the aqueous phase II did not affect transport rates at the initial stage (<12 h).

The substrate specificity of active transport systems was similar to that of the passive transport systems (see Table IV): for carrier **1**, Bz-Gly > Bz-Ala > Bz-Glu ~ (Bz-Gly-Gly) > Bz-Met > Bz-Val > Bz-Leu > Bz-Phe; for carrier **3**, Bz-Gly-Gly > Bz-Glu ~ Bz-Ala > Bz-Gly > Bz-Val > Bz-Met ~ Bz-Leu > Bz-Phe; for carrier **8**, Bz-Ala > Bz-Gly > Bz-Glu ~ Bz-Gly-Gly ~ Bz-Val > Bz-Met ~ Bz-Leu > Bz-Phe. The order of carrier capabilities in the active transport systems was in good accord with the expectations based on the results of passive transport systems: carrier **8** > carrier **1** > carrier **3**. It was strongly suggested that a variety of neutral ligand–metal complexes may transport amino acid anions against their concentration gradients and that their transport properties can be expected from those of passive transport systems.

In order to examine the nature of present active transport systems, we studied the extraction and releasing profiles for amino acid anions by using carriers **1**, **3**, and **8**, as described previously. The results are illustrated in Figure 4, in which the actively transported amounts of each amino acid anion are also included for comparison.

Their extraction behaviors were slightly but definitely varied by addition of chloride anion in the aqueous phase. While the extracted amounts of amino acid anions into the methylene chloride phase were suppressed in the presence of chloride anion, their extraction selectivities were enhanced. In particular, some amino acids with higher hydrophobicities such as Bz-Phe, Bz-Leu, and Bz-Val were effectively extracted into the membrane phase. These extraction trends in the presence of chloride anion were expectedly parallel to their transport selectivities as mentioned above, showing that the substrate-releasing process would play a key role in the present carrier system.

Recently some marocyclic polyethers were found to successfully mediate the active transport of amino acid derivatives, coupled with potassium cation transport.[8c] In such systems, the amino acid anions with higher hydrophobicities allowed fast transport: Bz-Phe > Bz-Leu ~ Bz-Met > Bz-Val > Bz-His ~ Bz-Ala ~ Bz-Gly-Gly ~ Bz-Gly. This transport trend also indicates that anion extraction into the membrane by complexation with potassium–polyether cation complex carrier would be importantly operating, in contrast with transition-metal complex carrier systems. Thus, transport selectivity could be dramatically modified and controlled by alterations of the carrier type used.

The present study demonstrates that transition metal complex mediated transport phenomena mainly depended on the coordination character of the metal complexes employed. Therefore, more adequate design of the metal complex carrier[17] can allow specific artificial transport of a variety of biologically important and interesting substrates such as amino acid, peptide, ATP, and other organic anions. At the same time, we could offer a new chemical analogue to the biological transport systems in which metal ions are significantly involved.[18]

## Experimental Section

**Carrier Synthesis.** The functionalized ligand used having the structure $[CH_2CH_2N(CSNHPh)]_{n=8}$ was prepared by ring-opening oligomerization of 1-(*N*-phenylthiocarbamoyl)aziridine in 70–80% yields.[10] Under an argon atmosphere, to an aziridine monomer solution in dried ethyl acetate (0.2 mol/L) was added dropwise with stirring an equimolar amount of diethyl sulfate at ambient temperature. After the resulting solution was allowed to stand at 60 °C for 20 days, oily viscous product, deposited from the reaction solution, was isolated, washed with ethyl

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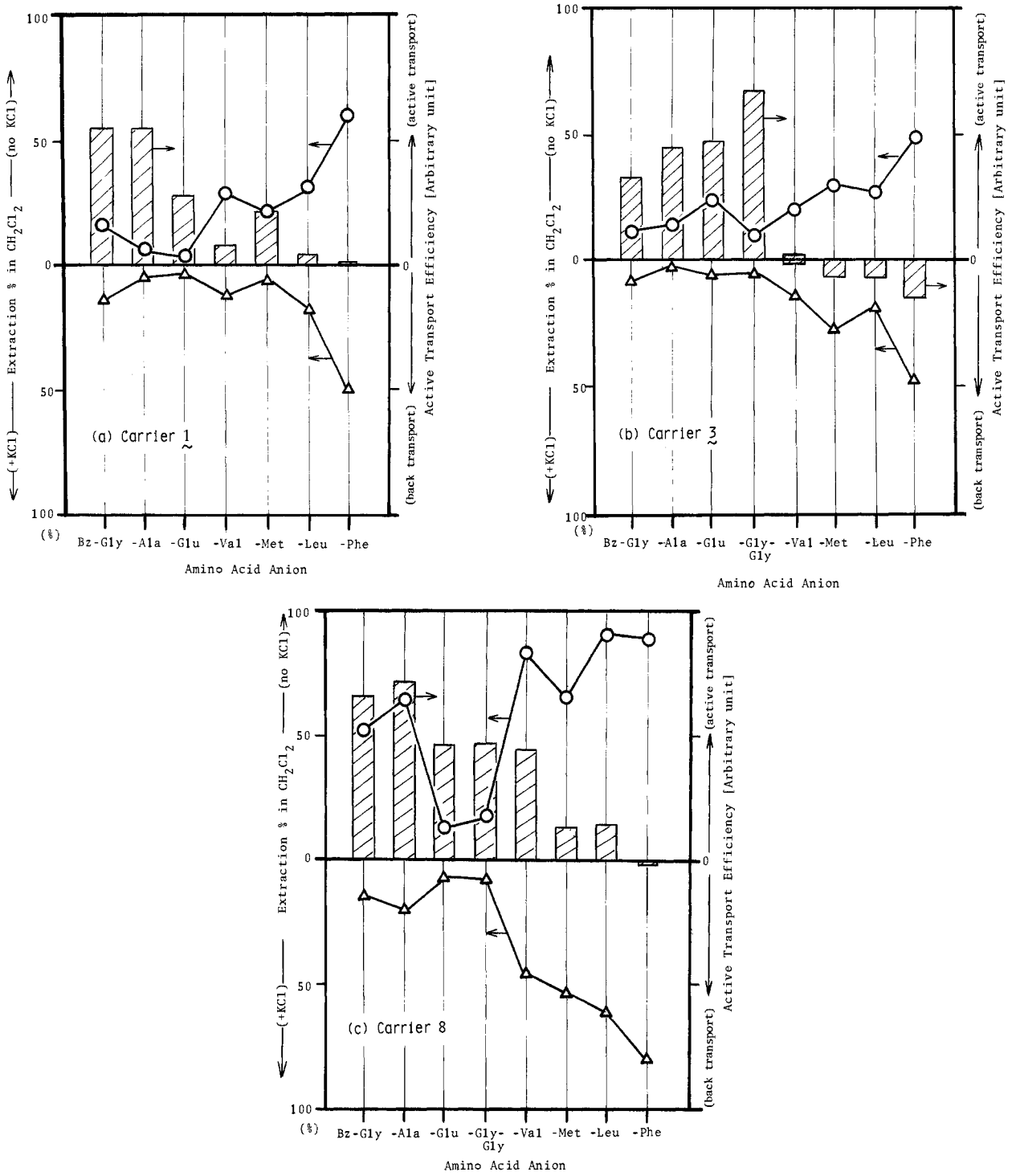


**Figure 4.** Relationship between extraction ability and active transport efficiency. Extraction conditions: carrier (**1**, **3**, or **8**), 0.0279 mmol/$CH_2Cl_2$, 4 mL; amino acid, 0.0186 mmol; KCl, 0 or 0.80 mmol/0.02 N aqueous NaOH, 4 mL. Transport conditions: see Table V.

acetate, and reprecipitated from tetrahydrofuran solution by adding ether. The obtained oligomer was immersed in 10% aqueous ammonia solution overnight, collected, washed with water repeatedly, and dried in vacuo. The crude white powder was purified by Amberlite 400 column chromatography. The oligomer was found to be an octameric material including a trace amount of analogues ($n$ = 7 and 9) by gel permeation chromatographic analysis. It is necessary to use the monomer immediately after preparation from aziridine and phenyl isothiocyanate. Otherwise, white insoluble product is formed instantaneously by adding diethyl sulfate. This white material is not available. The detailed properties of this oligomeric ligand have already been reported in the preceding papers.[10]

Copper complex **1** was obtained by mixing the octameric ligand (0.15 unit mmol), as prepared above, and copper(II) chloride (0.037 mmol) in 20 mL of methanol–methylene chloride solution (1:1, v/v) at room temperature for 30 min. After removal of solvent, the remaining green complex was dissolved in 8 mL of methylene chloride and used without further purifications. The IR spectrum of copper complex **1** exhibited some characteristic profiles in the –CSNH– frequencies: 1703, 1544, and 1440 $cm^{-1}$ (newly appeared bands), 1314 and 1227 $cm^{-1}$ (shift of bands). The complexation caused significant changes in the vibrational modes involved in the –CSNH– groups. A far-IR spectrum provided more direct information on the coordination states. Newly appeared bands at 300 and 252 $cm^{-1}$ could be ascribed to $\nu_{Cu-S}$ and the band at 112 $cm^{-1}$ to the $\delta_{S-Cu-S}$ mode. In the electronic spectrum of this complex in methylene chloride solution, the d–d transition absorption was observed at 710 nm. The coordination number was calculated as four from the plots of d–d transition absorbance against the mole ratio of the NCSNH unit to copper ion. An equilibrium constant was also estimated to be more than 3000 L/mol. Its preliminary cyclic voltammgram showed only

three reversible waves, suggesting that it has a uniform and simple structure and excellent stability in the electrochemical sense. A relevant study of the copper complex derived from the optically active octameric ligand $[CH_2CH(CH_3)N(CONHPh)]_{n=8}$[10c,19] showed typical copper bands with diffused fine structures, corresponding to essentially "square-planar" configuration in the CD spectrum.

The iron(III) complex **2** was similarly prepared by mixing octameric ligand (0.19 unit mmol) and iron(III) chloride (0.030 mmol) in 30 mL of methanol–methylene chloride solution (1:1, v/v) for 4 h. After removal of solvent, the methylene chloride soluble fraction (ca. 85%) was used. This complex was yellow red but exhibited no discrete absorption maximum in the methylene chloride. Although its coordination chemistry is not clear at present, it shows highly lipophilic properties, enough to be soluble in the used liquid membrane.

Other copper complexes **3**–**7** were synthesized according to literature methods.[20]

**Transport Experiments.** The passive transport experiments (Tables I and II) were carried out at room temperature (ca. 15 °C) in a similar apparatus as described before.[13] A cylindrical glass cell (4.0 cm, i.d.) holds a glass tube (2.0 cm, i.d.) that separates two aqueous phases. Typically, the inner aqueous phase (aqueous phase I) contains substrate anion in 4 mL of alkaline solution. The outer phase (aqueous phase II) contains antiport anion in 10 mL of water. The membrane layer (8 mL of methylene chloride), in which carrier is dissolved, lies below these two aqueous phases and bridges the separation by the central glass tube. This methylene chloride layer is stirred by a magnetic stirrer. Although we confirmed that the variations in stirring speed had no pronounced effect on the transport rates, the transport phenomena were not found to occur without any perturbations.

A similar transport experiment with each substrate anion was performed in the absence of carrier for reference, and leakage of substrate was found to be very small ($\sim 0.1 \times 10^{-6}$ mol/h).

The substrate anion concentration in aqueous phase II was confirmed to increase linearly with running time (<16 h), and the initial rates are shown in the tables.

The antiport anion was also observed to move from aqueous phase II to I. When the perchlorate anion was employed as antiport anion, we found that the amount of perchlorate anion transported, determined by the methylene blue method,[21] was almost equal to that of substrate anion transferred under the conditions stated in Tables I and IV. The detailed conditions are included in each table.

The active transport of amino acid derivatives was performed in a U-shaped glass cell (2.0 cm, i.d.). The carrier in methylene chloride (12 mL) is placed in the base of the cell, and two aqueous phases (5 mL each) of equal substrate concentration and pH are placed in the arms of the cell, floating on the methylene chloride. The membrane phase is stirred constantly by a magnetic stirrer.

When the salt of antiport anion (KCl, $NaClO_4$, or KSCN) was added in aqueous phase II, substrate anion was transported from aqueous phase I to II. The concentration of substrate anion in aqueous phase I increased with running time and reached a steady state (usually after 20 h). The concentration of substrate anion in aqueous phase I also decreased at an almost similar rate.

**Liquid–Liquid Extraction Experiments.** The methylene chloride solution of carrier was allowed to contact an aqueous solution of sodium salt of substrate anion. After a given period (ca. 1 h), an organic phase was separated from aqueous phase. The extracted amount of substrate anion was calculated from the difference between the concentrations of initially and remaining substrate anion in the aqueous phase. The detailed conditions are included in Table II.

**Registry No. 3**, 55997-76-7; **4**, 82469-60-1; **5**, 82469-61-2; **6**, 46369-53-3; **7**, 10380-28-6; **8**, 5137-55-3; $[CH_2CH_2N(CSNHPh)]_n$, 71093-49-7; copper(II) chloride, 7447-39-4; iron(III) chloride, 7705-08-0.

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# Proton NMR Characterization of the State of Protonation of the Axial Imidazole in Reduced Horseradish Peroxidase

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**Abstract:** The detection of a single proton exchangeable resonance for unligated ferrous horseradish peroxidase, HRP, with hyperfine shift comparable to that found for the proximal histidyl imidazole $N_\epsilon H$ in deoxy myoglobins, hemoglobin, and models established that the axial imidazole in ferrous HRP, contrary to the interpretations of other spectroscopic evidence, is not deprotonated. A protein conformational change modulated by another titratable residue induces a significant change in the $N_\epsilon H$ contact shift. We show that simultaneous consideration of changes of the proximal histidyl imidazole $N_\epsilon H$ contact shift and resonance Raman $\nu(Fe-N_\delta)$ permit the differentiation between steric and electronic influences on iron–imidazole bonding. For ferrous HRP, the acid → alkaline transition involves primarily changes in $N_\epsilon H$ hydrogen bonding to a peptide acceptor, with the degree of imidazolate character for the axial ligand slightly larger for the acid than for the alkaline form of the protein.

The proposal that electronic control of iron reactivity in hemoproteins is exercised primarily through the axial ligand is a cornerstone of the theories for the mechanism of action of both hemoglobins and heme peroxidases. The original Perutz model for hemoglobin cooperativity emphasized strained or stretched iron–histidine bonds.[1] More recently, recognition has been given to the fact that the strength of the iron–imidazole bond can be modulated indirectly by the formation of a hydrogen bond between the proximal histidyl imidazole $N_\epsilon H$ (A in Figure 1) and a protein acceptor residue.[2-6] Increased donor properties of the $N_\epsilon H$ would increase the imidazole $\sigma$ basicity and hence strengthen the iron–imidazole bond.[3] Even complete deprotonation of the imidazole in hemoglobin has been considered on the basis of ESR data,[3,4]

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